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Crystallization and preliminary X-ray analysis of brazzein, a new sweet protein

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Abstract

Brazzein is a sweet protein isolated from a wild African plant *Pentadiplandra brazzeana*. Brazzein is the smallest (molecular mass = 6473 Da) and the most water-soluble protein sweetener discovered so far and is highly thermostable. Crystals were grown by vapor diffusion using sodium sulfate as a precipitant. They belong to the tetragonal space group $I4_122$ with unit-cell parameters $a = b = 61.4$, $c = 59.6$ Å and with one molecule in the asymmetric unit. The crystals diffract to 1.8 Å resolution using synchrotron radiation.

1. Introduction

Some proteins are known to stimulate taste receptors and elicit sweet tastes (Kurihara & Nirasawa, 1994). To date, six proteins, monellin (Morris & Cagan, 1972; van der Wel, 1972; Frank & Zuber, 1976), thaumatin (van der Wel & Loeve, 1972; Iyengar *et al.*, 1979), pentadin (van der Wel *et al.*, 1989), curculin (Yamashita *et al.*, 1990; Harada *et al.*, 1994), mabinlin (Liu *et al.*, 1993; Nirasawa, Liu, Nishino & Kurihara, 1993) and brazzein (Ming & Hellekant, 1994) have been reported to function as sweeteners. In addition to its intrinsic sweet taste, curculin is able to transform a sour taste into a sweet taste. Miraculin (Theerasilp & Kurihara, 1988; Theerasilp *et al.*, 1989; Takahashi, Hitotsuya, Hanazawa, Arata & Kurihara, 1990) is not sweet in itself but possesses a similar taste-modifying activity. These sweet proteins and sweetness-inducing proteins have been used to sweeten foods and drinks in the regions in which the source plants are endemic.

Brazzein consists of 54 amino acids with four disulfide bridges (Fig. 1, Ming & Hellekant, 1994; Kohmura *et al.*, 1996). The molecular weight is 6473 Da, which is much smaller than that of any other sweet protein discovered to date, such as monellin (12 491 Da) and thaumatin (22 206 Da). Sensory analysis shows that brazzein is 2000 times sweeter in comparison to 2% sucrose, and has a more phasic response and a faster adapting tonic phase than thaumatin (Ming & Hellekant, 1996). Brazzein is the most water soluble of the known sweet proteins, and its sweetness remains even after incubation at 353 K for 4 h (Ming & Hellekant, 1994), probably because its four disulfide bridges result in a tightly folded structure.

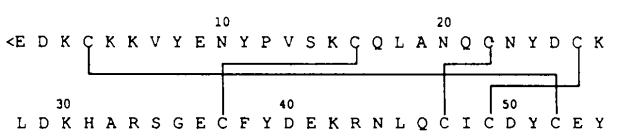

Fig. 1. Amino-acid sequence of brazzein showing the disufide bridge assignment. <E represents pyroglutamic acid.

To date, the crystal structures of two sweet proteins, monellin and thaumatin, have been determined (Ogata, Hatada, Tomlinson, Shin & Kim, 1987; de Vos *et al.*, 1985) and recently refined at 2.75 and 1.6 Å, respectively (Ogata, Gordon, de Vos & Kim, 1992; Somaza *et al.*, 1993). In addition, single-chain monellin, which was genetically engineered by fusing the two chains of monellin into a single 94-residue polypeptide, was crystallized and its crystal structure was determined at 1.7 Å resolution (Ogata *et al.*, 1992). These refined high-resolution structures allowed detailed structural comparisons between monellin and thaumatin; however, no obvious structural similarities have been discovered. Thus, the active sites which interact with the sweet taste receptors have not yet been identified. Since brazzein is a smaller protein, its crystal structure may provide more direct clues about the sweet determinant and may lead to the production of new low-molecular-weight sweeteners.

2. Experimental

Brazzein was obtained from the fruits of *Pentadiplandra brazzeana*, according to the methods of Ming & Hellekant (1994). A sample was further purified by high-performance liquid chromatography (HPLC). Preparative HPLC was performed on a Waters Prep LC 3000 System Controller under gradient conditions [GL Sciences Inertsil ODS column, 5 μm, 20 × 250 mm; eluting solvents: *A*, 0.05% trifluoroacetic acid (TFA): *B*, acetonitrile containing 0.05% TFA]. The protein (30 mg) was dissolved in 6 ml of a mixture (*A*:*B* = 9:1) of the solvents, loaded onto the column, and eluted with a linear gradient of 10% *B* to 18% *B* obtained in 55 min, 18% *B* to 25% *B* in 65 min, and 25% *B* to 10% *B* in 75 min at a flow rate of 10 ml min^{-1}. The protein fractions were collected, concentrated and lyophilized.

Crystallization was performed using sodium sulfate as a precipitant by the hanging-drop method. Crystals were grown by mixing equal volumes of 20 mg ml^{-1} protein with 1.1–1.2 *M* sodium sulfate, 0.1 *M* sodium citrate (pH 4.0) and equilibrating against a reservoir containing the same solution at 293 K. Thin rod-shaped crystals appeared in a period of 1–2 weeks. Then, the macroseeding technique was applied to obtain crystals suitable for X-ray crystallographic studies. A seed crystal was introduced into the hanging drop which was made by mixing an equal volume of 10–15 ml min^{-1} protein with 0.9–1.0 *M* sodium sulfate, 0.1 *M* sodium citrate (pH 4.0), and then the drop was equilibrated against a reservoir containing the same solution. Higher concentrations of the protein and the precipitant had a tendency to cause branching of the seed crystal. Crystals grew to a maximum size of 0.15 × 0.2 × 1.0 mm within a few weeks.

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3. Results and discussion

Examination of these crystals using a Rigaku R-AXIS IIc imaging-plate detector with a Rigaku RU200 rotating-anode generator showed that they were of space group $I4_122$ with unit-cell dimensions of $a = b = 61.4$, $c = 59.6$ Å. Assuming one molecule per asymmetric unit, the calculated packing density of the crystal is $V_m = 2.17\ \text{Å}^3\ \text{Da}^{-1}$. The corresponding solvent content is 43%(*v*/*v*), which is well within the range normally found for proteins (Matthews, 1968).

X-ray data to 1.8 Å resolution were collected using the macromolecular-oriented Weissenberg camera (Sakabe, 1991) at beamline 6A2 of the Synchrotron Radiation Source at the National Laboratory for High Energy Physics (Photon Factory), Tsukuba. The wavelength was set to 1.04 Å and a beam collimator of 0.1 mm was used. Data evaluation was performed with the program *WEIS* (Higashi, 1989). The merging $R(I)$ factor ($\Sigma_i|I_i - \langle I_i\rangle|/\Sigma_i I_i$, where $\langle I_i\rangle$ is the average of I_i over all symmetrical equivalents) was 0.076 for 6015 unique reflections [$I > 1.0\sigma(I)$] obtained from 20 686 measurements. The data set was 89.4% complete to 1.8 Å resolution. The completeness in the resolution shell between 1.88 and 1.8 Å was 74.9% and the $\langle F\rangle/\langle\sigma(F)\rangle$ value in this range was 2.52.

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